Association between the proportion of *Plasmodium falciparum* and *Plasmodium vivax* infections detected by passive surveillance and the magnitude of the asymptomatic reservoir in the community: a pooled analysis of paired health facility and community data

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Summary

Background Passively collected malaria case data are the foundation for public health decision making. However, because of population-level immunity, infections might not always be sufficiently symptomatic to prompt individuals to seek care. Understanding the proportion of all *Plasmodium* spp infections expected to be detected by the health system becomes particularly paramount in elimination settings. The aim of this study was to determine the association between the proportion of infections detected and transmission intensity for *Plasmodium falciparum* and *Plasmodium vivax* in several global endemic settings.

Methods The proportion of infections detected in routine malaria data, P(Detect), was derived from paired household cross-sectional survey and routinely collected malaria data within health facilities. P(Detect) was estimated using a Bayesian model in 431 clusters spanning the Americas, Africa, and Asia. The association between P(Detect) and malaria prevalence was assessed using log-linear regression models. Changes in P(Detect) over time were evaluated using data from 13 timepoints over 2 years from The Gambia.

Findings The median estimated P(Detect) across all clusters was 12·5% (IQR 5·3–25·0) for *P falciparum* and 10·1% (5·0–18·3) for *P vivax* and decreased as the estimated log-PCR community prevalence increased (adjusted odds ratio [OR] for *P falciparum* 0·63, 95% CI 0·57–0·69; adjusted OR for *P vivax* 0·52, 0·47–0·57). Factors associated with increasing P(Detect) included smaller catchment population size, high transmission season, improved care-seeking behaviour by infected individuals, and recent increases (within the previous year) in transmission intensity.

Interpretation The proportion of all infections detected within health systems increases once transmission intensity is sufficiently low. The likely explanation for *P falciparum* is that reduced exposure to infection leads to lower levels of protective immunity in the population, increasing the likelihood that infected individuals will become symptomatic and seek care. These factors might also be true for *P vivax* but a better understanding of the transmission biology is needed to attribute likely reasons for the observed trend. In low transmission and pre-elimination settings, enhancing access to care and improvements in care-seeking behaviour of infected individuals will lead to an increased proportion of infections detected in the community and might contribute to accelerating the interruption of transmission.

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Introduction

For diseases such as malaria, infections do not always lead to clinical manifestations and clinical symptoms might not be caused by the parasitic infection. Thus, passive case detection (PCD) data will underestimate the true magnitude of infections.[1–3] Despite efforts to ensure that all confirmed care-seeking malaria infections are captured as part of PCD, little is known about the potential implications of uncounted asymptomatic infections on estimates of malaria infections. This undercounting could help to explain why global estimates of malaria vary substantially depending on how these hidden infections are counted.[4] Accounting for asymptomatic infections is especially important in malaria elimination settings: targeted interventions might be implemented before transmission is low enough for them to be effective, and

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Research in context

Evidence before this study

A strong surveillance system is a core intervention in the global strategy for malaria control and elimination. Despite recent progress in strengthening the quality of the data generated in health systems, research suggests that a sizeable population of individuals with asymptomatic infections who do not seek medical care are present in most endemic settings. By not accounting for these infections, malaria burden is underestimated. The probability that an individual becomes symptomatic for *Plasmodium falciparum* malaria is a function of the protective immunity acquired over repeated exposure to malaria. Therefore, the magnitude of the potential undercounting in estimates of malaria burden that rely on routinely collected data would likely be a function of transmission intensity, whereby the effectiveness of the health system in detecting malaria infections should improve as transmission intensity and population-level immunity to malaria decrease. However, previous studies have not been able to directly assess this issue outside of modelling frameworks. Based on a search of the PubMed and Embase databases in English and French from inception to Dec 31, 2018, using general search terms "malaria", "epidemiology", and "polymerase chain reaction [PCR]", no database of paired community survey and health systems for either *Plasmodium falciparum* or *Plasmodium vivax* data covering a range of transmission intensities exists.

Added value of this study

Our work has created a database consisting of 431 and 213 paired PCR prevalence and clinical incidence data covering 13 and seven countries for *P falciparum* and *P vivax*, respectively, in three endemic regions. We found that, on average, health systems detect only a small fraction of all infections, with the heath system effectiveness improving at the lowest range of transmission intensity. Factors associated with an improved proportion of infections detected included being in the high transmission season, smaller catchment population sizes, care-seeking behaviours, and a recent change of transmission as a proxy for the expected levels of population-level protective immunity.

Implications of all the available evidence

Our results are the first data estimating the proportion of malaria infections expected to be symptomatic and seek care and the potential magnitude of the undercounting associated with asymptomatic infections in quantifying malaria transmission intensity. The evidence suggests that the health system becomes more effective at detecting malaria infections once transmission intensity is sufficiently low. Where the goal is to eliminate malaria transmission, the notion that the health system becomes more effective at detecting infections when transmission is low is reassuring. Improving access to care for testing and promoting better care-seeking behaviour of infected individuals would lead to more infections being detected. For settings accelerating malaria elimination, this confidence that any infections are likely to be sufficiently symptomatic to seek care becomes especially relevant whereby any residual population-level protective immunity has the potential to mask any lingering or introduced infections and could lead to programmes failing to sustain interruption of transmission.

any residual infections provide a source for onward transmission.[5,6]

Asymptomatic malaria infections are common in endemic areas; patients with such infections are not expected to seek care and consequently the infections are not detectable by malaria surveillance activities.[7,8] The presence and persistence of asymptomatic infections is a complex phenomenon related to levels of protective immunity acquired with repeated exposure to malaria and the maturity of the immune system.[9] If an individual is not sufficiently symptomatic to prompt care seeking or if parasite densities are not sufficiently high to be detected using rapid diagnostic tests or microscopy—the routinely used diagnostics for confirming malaria infections in people with clinical symptoms—they cannot be detected within routine aggregation of PCD data as part of malaria surveillance activities.[10] Understanding the magnitude of undercounting, and if or when all infections in a community are expected to become symptomatic and therefore passively detectable, becomes paramount for settings aiming to achieve malaria elimination.

We did a pooled analysis of paired cross-sectional household surveys and routinely collected PCD data for both *Plasmodium falciparum* and *Plasmodium vivax* to assess the impact of asymptomatic infections on the interpretation of malaria surveillance data and factors affecting any associations with transmission intensity. The relation between the proportion of all infections detected in health systems—P(Detect)—and the parasite reservoir in the community as estimated by PCR, used here as a proxy for transmission intensity and the expected levels of protective immunity in the population,[9] and any changes over time were examined.

Methods

Literature review and data collection

A literature review was done by GS using the search terms "Plasmodium" AND "cross sectional survey" to identify community-based cross-sectional household surveys for *P falciparum* infection in which data collection was done either at a single or multiple timepoints. Any potentially eligible study identified in PubMed or Embase, irrespective of publication date, was assessed for eligibility. We sought studies that reported or could extrapolate all-age malaria prevalence in the community, diagnosed either via molecular methods or estimated from rapid diagnostic test data using validated methods.[8,11–13] Studies were excluded if any of the following criteria were met: the sampled population was not comprised of randomly selected households; only a subset of individuals residing in the selected

households were tested for malaria; the passive surveillance data from health facilities could not be obtained within the timeframe of the study; or it was not possible to match the individuals represented in the health facility and community survey data in geographical space and therefore represent the same target population (appendix pp 1–2). If the community data were available in the required format, the study authors were contacted to request access to the data and to ask whether the paired health facility data were available and could be accessed. Searches were limited to abstracts or titles written in English or French.[14] Unpublished data were also sought by asking investigators for studies that met our inclusion criteria, which consisted of any cross-sectional surveys using randomly selected household as the sampling unit.

Household survey data

For the household survey data, access to the individual-level community survey data was requested from the corresponding authors. The primary focus was on *P falciparum*, but *P vivax* data were gathered when available. The data collected from the household survey were used both to estimate the total number of infected individuals likely to be within the catchment population and the current transmission intensity. Alternative methods for determining transmission intensity are possible, but all methods lead to consistent estimates; for this study, we used prevalence because it was available for all clusters.[15,16] The variables requested were malaria test result, diagnostic method, age, sex, geographical coordinates for the household, survey date, recent malaria control interventions in the population, and total population size. In cases where the same population was sampled at different timepoints, baseline data were included as well as data from subsequent surveys representing different periods of seasonal transmission, with the high malaria season typically associated with periods of intense rains.

PCD data

The existence of PCD data already linked to the community surveys was not an essential criterion for inclusion. If not available as part of the study design, the ability to retrospectively extract the required PCD data from the relevant facilities or existing databases was required. Variables collected from the routinely reported PCD data included the number of confirmed malaria cases reported over the time period matching the community survey, diagnostic method, and georeferencing information for patient households where needed to match the two populations. Any imported cases, as classified according to the local guidelines, were excluded from the analysis because such cases do not represent local transmission. Any case identified in the week before and after completion of the community-based data collection was included to account for any delays in treatment seeking or the intrinsic incubation period whereby an infection in the community can represent a transmission event occurring within 1 week before or after data collection.

Spatial matching was done to ensure comparability between the populations under analysis. The community survey design and availability of routine data on facility catchment boundaries determined the approach (appendix p 1). For most studies, catchment areas were overlapping and therefore no data adjustment was necessary. For the single study with non-overlapping populations and for which spatial information was available, geolocation data were extracted from the health facility registers for each case, which were collected as part of a concurrent cluster randomised trial.[6] For any studies identified in the literature where this match was not good and geolocation information was not available, the site was excluded. Using the available information, populations for both datasets were restricted to those overlapping in space (appendix p 2). The number of confirmed cases in the populations younger than or older than 5 years of age, as defined by the routine aggregation of malaria data, was also collected where available.[1]

Covariate data

Covariates of interest included reported insecticide-treated bednet use the previous night, whether or not the house had been treated with indoor residual spray in the past 12 months, self-reported health care-seeking behaviour for fevers, and any recent study-implemented intervention that had taken place. Covariate data were obtained from the cross-sectional surveys if available. When these surveys were not available, data were extracted from the geostatistical models developed by the Malaria Atlas

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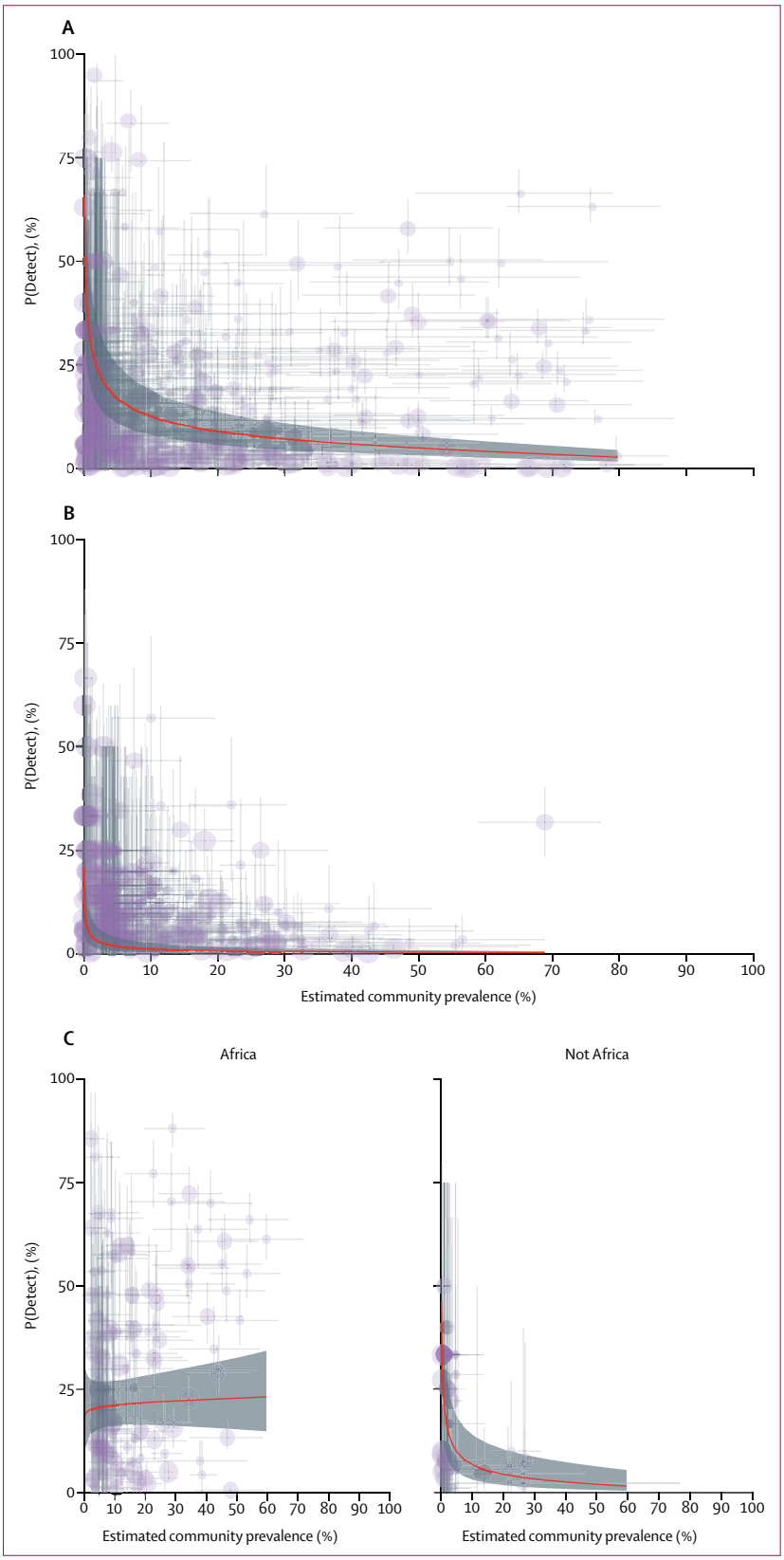
	P falciparum (clusters, n)			*P vivax* (clusters, n)		
	All ages	Individuals older than 5 years	Children aged 5 years and younger	All ages	Individuals older than 5 years	Children aged 5 years and younger
Brazil	6	6	6	6*	6*	6*
Cambodia	34	20	20	7	7	..
Ethiopia	1	..	..	1	..	..
The Gambia	36	34	34	..	..	..
Haiti	14	14	10	..	..	..
Kenya	10	9	9	..	..	..
Laos	17	17	17	17	17	17
Malaysia	7	6	6	7	..	..
Myanmar	199	199	..	171	171	..
Peru	7	..	..	..	..	..
Philippines	4	..	..	4	..	..
Tanzania	25	4	4	..	..	..
Zambia	111	..	95	..	..	
Total	471	309	201	213	201	23

The table shows the clusters for which data from all ages as well as data focusing only on those older than 5 years of age or children 5 years of age and younger were available for analysis. Studies covered the period from 2008 to 2017. *Two of the clusters are different from those reporting *P falciparum*.

Table 1: **Numbers of paired community survey and health facility clusters available for both the *Plasmodium falciparum* and *Plasmodium vivax* analysis in each country**



Project (MAP) from the time and location of data collection.[13,17] If gaps remained, expert opinion was sought from the study investigators. Recent changes in transmission intensity in the studied population were quantified by using the predicted annual *P falciparum* incidence according to the MAP models for the year and location of the survey. The difference in *P falciparum* incidence between the study year and that estimated 1 year and 5 years previously was calculated and included as a covariate to capture any potential mismatch between current transmission intensity and expected population-level protective immunity. These data were not available for *P vivax*.

Ethics approval for all secondary use of data and collection of passively collected data was obtained from the London School of Hygiene & Tropical Medicine (ethics approval number 14320). If required, additional local ethics approval was also obtained for this specific analysis (ethics approval numbers RITM-170818, UnzaRec-1131, NBC Haiti-1516-29, and 1617-31). Approvals for the original data collection are reported in the site-specific publications (appendix pp 3–4).

Derivation of P(Detect)

The main outcome of interest was P(Detect) per study cluster, wherein a cluster consists of a paired health facility and community population. Data used to derive the estimates included the number of cases detected at the health facility, the number of people infected, the number sampled in the community survey, and the total population of the community. Mixed infections identified were included in the species-specific analysis, but too few were reported to be assessed as a separate outcome. Briefly, to account for the zero-inflated distribution of case counts, Bayesian methods based on a Polya Urn model for finite populations were used to estimate P(Detect) and corresponding levels of uncertainty. The simulation algorithm was implemented in the R software (version 3.3) using the polyapost package (appendix pp 5–7).

Longitudinal data

Data from 13 monthly surveys over 2 years collected in The Gambia were used to assess seasonal trends in P(Detect).[18] P(Detect) was estimated in each study village for each month of data collection. The resulting estimates

Figure 1: **Estimated proportion of *Plasmodium falciparum* infections in populations detected within health systems (P[Detect]) compared with the corresponding prevalence of infection in the community**
(A) All age groups. (B) Individuals older than 5 years of age. (C) Children aged 5 years and younger, with the significant interaction in non-African and African populations shown in the separate panels. The average fitted linear mixed model trend is shown by the red line and corresponding 95% CI band is shaded in grey. Each dot represents a paired community and health facility cluster, with their size representing the sample size of the community survey as small (<50 people), medium (50–100 people), or large (>150 people). The 95% credible intervals around each metric are shown by the horizontal and vertical grey lines around each cluster.

	All ages (n=471 clusters)		Individuals older than 5 years (n=309 clusters)		Children aged 5 years and younger (n=201 clusters)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Intercept	3·80 (1·65–8·73)	0·0021	0·01 (0·006–0·02)	<0·0001	0·36 (0·22–0·58)	0·0001
Log odds community prevalence	0·63 (0·57–0·69)	<0·0001	0·58 (0·53–0·64)	<0·0001	1·04 (0·89–1·22)	0·61
Non-African region (*vs* African countries)	0·37 (0·22–0·62)	0·0003	4·45 (2·00–9·89)	0·0004	0·08 (0·02–0·29)	0·0002
Log_{10} population size	0·23 (0·17–0·31)	<0·0001	..	..	..	..
Low transmission season (*vs* high transmission system)	0·59 (0·46–0·77)	0·0001	0·65 (0·53–0·80)	0·0001	0·62 (0·44–0·87)	0·0067
RDT used as community diagnostic (*vs* PCR)	4·27 (2·31–7·90)	<0·0001	0·07 (0·03–0·14)	<0·0001	..	..
Increase in malaria incidence in the previous year	431·82 (2·07–89859·3)	0·028	..	..	..	..
Log odds p (seek care if febrile)*	..	..	0·71 (0·58–0·87)	0·0015	0·85 (0·71–1·01)	0·075
Log odds community prevalence: region	..	..	..	..	0·55 (0·42–0·73)	0·0001
Log odds bednet use	..	..	2·29 (1·16–2·37)	0·024	..	..

Detection of infection in the full all-age population, in the populations aged older than 5 years, and in children aged 5 years and younger is shown. Some cells are empty because the factor was not retained in the adjusted analysis because they did not contribute to the explanatory power of the model. OR=odds ratio. RDT=rapid diagnostic test. *The probability of patients seeking care if febrile is the proxy variable typically used in malaria research to provide a proxy estimate for treatment seeking.

Table 2: **Fixed-effects results of the mixed-effects log-linear regression for the proportion of *Plasmodium falciparum* infections detected in the health system according to community-level transmission intensity**

were plotted over time with the locally estimated scatterplot smoothing fit used to assess the average trend across all villages and stratified according to low (estimated PCR prevalence below 8·0%) or high transmission intensity (above 8·0%).

Statistical analysis

Log-linear regression was conducted with the lme4 package for the R software. The dependent variable was the logit of P(Detect) with a Gaussian family and the independent variable was community-level PCR prevalence. Random-effects models were used to account for the effect of the study cluster in the *P falciparum* surveys; these model fits resulted in minimal improvement for *P vivax* data, so fixed-effects models were employed for *P vivax*. Models were estimated according to the restricted maximum likelihood estimation method for computational efficiency. Covariates, interaction terms, and splines were tested with the best model fit ascertained according to the Akaike Information Criterion value. The log odds estimates were back-transformed to prevalence for ease of interpretation and the average model fit and corresponding uncertainty were plotted for visualisation.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Our search for published and unpublished studies resulted in data from 471 clusters in 13 countries for *P falciparum* and 213 clusters in seven countries for *P vivax* (table 1, appendix p 8). The malaria diagnostic used in most clusters was ultrasensitive PCR (in 250/471 for *P falciparum* and in 194/213 for *P vivax*), which has a limit of detection that is 50-times more sensitive than that of conventional PCR.[19] 132 clusters from two countries (Zambia [n=111] and Tanzania [n=21]) required adjustment because only rapid diagnostic test data were available.[8] PCD data representing individuals older than 5 years of age were available for 309 clusters for *P falciparum* and 201 clusters for *P vivax*, and data for children aged 5 years and younger in 201 clusters for *P falciparum* and 23 clusters for *P vivax*. The discrepancy in the number of clusters is due to clusters for which age adjustment was required being excluded from the age-specific analysis.

For *P falciparum* malaria, the cluster-level all-age estimated PCR prevalence was 16·17% (95% CI 14·43–17·91), ranging from 0·04% to 79·74%. The 471 clusters had a median size of 511 people (IQR 148–4724) but ranged from 24 to 100 000 individuals, with the fraction sampled between 0·03% (8/22 988) and 99·2% (392/418) of the population. The median estimated P(Detect) was 12·5% (IQR 5·3–25·0). There was a negative association between P(Detect) and estimated PCR prevalence in the community (adjusted odds ratio [OR] 0·63, 95% CI 0·57–0·69; figure 1A; appendix pp 9–10). Health facilities had lower odds of detecting infections in larger than in smaller communities (adjusted OR 0·23, 95% CI 0·17–0·31), during the low transmission season (0·59,

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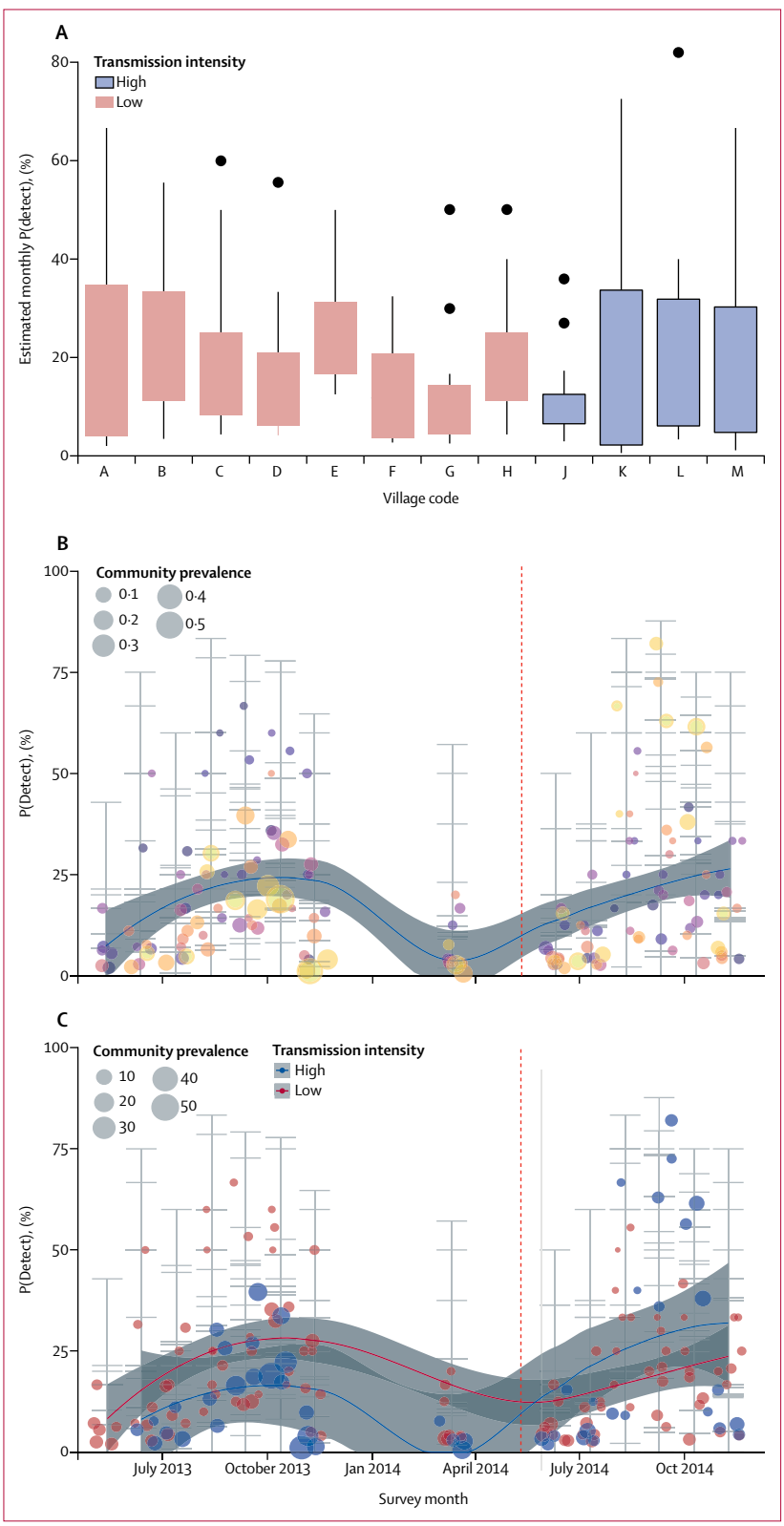
See **Online** for appendix



0·46–0·77), and in non-African compared with African settings (0·37, 0·22–0·62). Settings in which malaria incidence had increased in the year before the survey were more likely to have infections detected (adjusted OR 431·82, 95% CI 2·07–89859·3; table 2).

Within the 309 clusters with data on infections in those older than 5 years of age, a similar but more extreme trend was observed as that for the all-age population, whereby most infections remained unrepresented at the facility level until reaching the lowest levels of estimated PCR prevalence (figure 1B). In the population older than 5 years of age, health facilities had an increased odds of detecting infections in non-African settings compared with African settings (adjusted OR 4·45, 95% CI 2·00–9·89), and where there was a higher reported use of insecticide-treated bednets in the population (2·29, 1·16–2·37). There were lower odds of infections detected within health facilities during the low transmission season than in the high transmission season (adjusted OR 0·65, 95% CI 0·53–0·80; table 2).

The odds of detecting infections in health facilities in children aged 5 years and younger also showed a negative association with estimated community PCR prevalence, but there was a significant interaction by region (figure 1C). The relation in African settings showed a slight positive association with transmission intensity. By contrast, the relation in non-African settings was similar to that observed in the population older than 5 years of age, with an increase in P(Detect) once estimated prevalence was sufficiently low (figure 1C; appendix pp 9–10). Similar factors to the other models were associated with P(Detect) and the odds of detecting infections were lower during the low transmission season (adjusted OR 0·62, 95% CI 0·44–0·87; table 2).

The seasonal pattern in P(Detect) was specifically assessed using a longitudinal dataset with 12 villages in The Gambia with paired PCD and community data spanning 2 years. Annual village-level estimated PCR prevalence ranged from 2·29% to 24·18% (figure 2). Overall, monthly estimates of P(Detect) ranged from 0·57% to 82·05%, with the largest within-village variation observed in village L (ranging from 3·33% to 82·05%;

Figure 2: **Estimated proportion of *Plasmodium falciparum* infections in populations detected within health systems (P[Detect]) in 12 communities sampled at 13 monthly intervals over 2 years in The Gambia**
(A) The annual variation within each study village (A to M) is shown as a boxplot, with low transmission villages represented in orange and high transmission villages in blue. (B) The locally estimated scatterplot smoothing (LOESS) trends for all villages combined with the different colours representing the 12 individual villages. (C) The LOESS trends for villages stratified according to high transmission intensity (blue line) or low transmission intensity (orange line). The 95% CIs from the LOESS estimate are shown as the shaded grey area. The 95% credible intervals around P(Detect) are shown by the vertical grey lines around each, with the point size representing the estimated community prevalence for that sample month. The dashed vertical red line identifies the period where a mass drug administration of dihydroartemisinin–piperaquine was deployed in all study villages.[18]

figure 2A). A seasonal pattern whereby P(Detect) increases during the high transmission season (usually September–December) was evident (figure 2B). When stratified by villages with high (n=4) and low (n=8) transmission intensity, a similar seasonal pattern emerged (figure 2C).

For *P vivax* malaria, mean cluster-level all-age estimated PCR prevalence was 14·47% (95% CI 12·5–16·37) but ranged from 0·05% to 93·75%. The 213 clusters of paired data ranged in size from 24 to 20 841 individuals with the fraction sampled between 0·02% (82/4168) and 94·44% (51/54) of the population. The median estimated P(Detect) was 10·1% (IQR 5·0–18·3). Again, there was evidence of a negative association between P(Detect) and estimated PCR prevalence in the community (adjusted OR 0·52, 95% CI 0·47–0·57; figure 3A, appendix pp 9–10). There were lower odds of detecting infections in Asian than in non-Asian settings (adjusted OR 0·05, 95% CI 0·02–0·12) and lower odds of detecting infections in larger than in smaller populations (0·23, 0·17–0·32). P(Detect) was also likely to increase in communities in which ultrasensitive PCR was used as the diagnostic tool when compared with communities where malaria was assessed using other PCR methods (adjusted OR 4·09, 95% CI 2·12–7·90; table 3).

When examining the 201 clusters with data about *P vivax* infections in the population aged older than 5 years, a similar trend was observed to that in the all-age population, with most infections remaining undetected until the lowest estimates of estimated PCR prevalence (adjusted OR 0·51, 95% CI 0·47–0·56; figure 3B, appendix pp 9–10). Infections in those aged older than 5 years were more likely to be detected at health facilities where a recent intervention (mass drug administrations targeting *P falciparum* with or without concurrent long-lasting insecticidal net distribution) took place than in those without a recent intervention (adjusted OR 1·56, 95% CI 1·01–2·41), and where the probability of individuals seeking care if febrile was higher (1·95, 1·46–2·60). There were lower odds of infections being detected in larger than in smaller communities (adjusted OR 0·22, 95% CI 0·16–0·31; table 3). In the 23 clusters with data available for *P vivax* infections in children aged 5 years and younger, a similar trend to that seen in all ages as well as individuals older than 5 years was observed; however, the number of clusters was insufficient for further analysis (appendix p 11). Results of the validation tests for all models suggest a good predictive capacity (appendix pp 12–14).

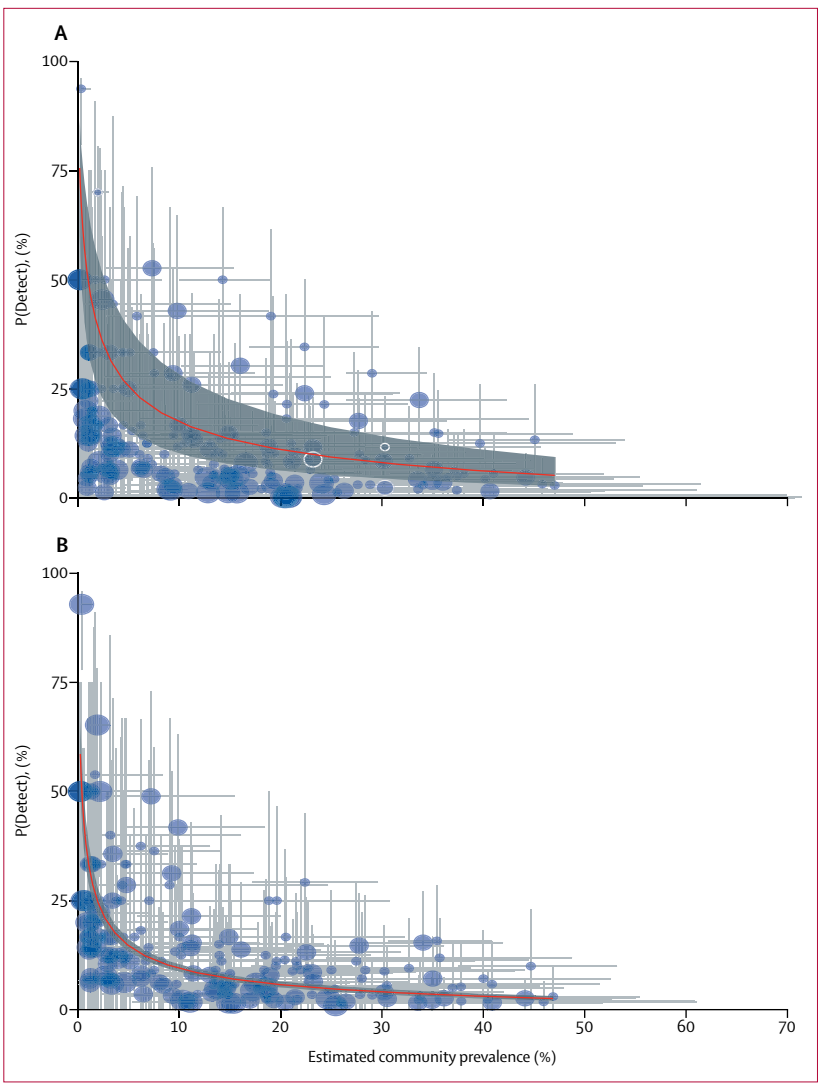


Figure 3: **Estimated proportion of *Plasmodium vivax* infections detected in health facilities compared with the corresponding prevalence of infection in the community**
(A) All age groups. (B) Individuals older than 5 years of age. The average fitted linear mixed model trend is shown by the red line and corresponding 95% CI band is shaded in grey. Each dot represents a paired community and health facility cluster, with their size representing the sample size of the community survey as small (<50 people), medium (50–100 people), or large (>150 people). The 95% credible intervals around each metric are shown by the horizontal and vertical grey lines around each cluster.

Discussion

The presence of asymptomatic infections across the malaria transmission spectrum has been well established but not adequately quantified.[8] Irrespective of issues related to health system capacity, such infections are not typically captured as part of routine passive surveillance. Using paired health facility and cross-sectional survey data, we have described the extent to which malaria is underestimated due to the prevalence of asymptomatic infections for both *P falciparum* and *P vivax* malaria when relying solely on malaria surveillance data, providing a proxy measure for the proportion of infections that are symptomatic enough for infected individuals to seek care. Crucially, we have shown how this association changes as transmission intensity decreases. Although for malaria control programmes to know the true transmission intensity will be difficult, as will ascertaining whether or not the clinical symptoms driving care-seeking are attributable to malaria, the observation that more infections are identified within the health facility once

	All ages (n=213 clusters)		Individuals older than 5 years of age (n=201 clusters)	
	Adjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Intercept	2·72 (0·94–7·92)	0·067	0·24 (0·11–0·54)	0·0006
Log odds community prevalence	0·52 (0·47–0·57)	<0·0001	0·51 (0·47–0·56)	<0·0001
Region: Asia (*vs* non-Asia)	0·05 (0·02–0·12)	<0·0001	..	..
Log_{10} population size	0·23 (0·17–0·32)	<0·0001	0·22 (0·16–0·31)	<0·0001
Community diagnostic: usPCR (*vs* other PCR)	4·09 (2·12–7·90)	<0·0001	..	..
Log odds bednet use	1·08 (1·00–1·18)	0·049	..	..
Recent intervention*	..	..	1·56 (1·01–2·41)	0·044
Log odds p (seek care if febrile)†	..	..	1·95 1·46–2·60)	<0·0001

Detection of infection in both the full all-age population and detection of infections in the population older than 5 years of age. Some cells are empty because the factor was not retained in the adjusted analysis because they did not contribute to the explanatory power of the model. OR=odds ratio. usPCR=ultra-sensitive PCR. *Mass drug administrations targeting *Plasmodium falciparum* with or without concurrent long-lasting insecticidal net distribution. †The probability of patients seeking care if febrile is the proxy variable typically used in malaria research to provide a proxy estimate for treatment seeking.

Table 3: **Fixed-effects results of the mixed-effects log-linear regression for the proportion of *Plasmodium vivax* infections detected in the health system according to community-level transmission intensity**

transmission is sufficiently low (eg, a higher estimated P[Detect]) is reassuring.[20]

The observed pattern for *P falciparum* is consistent with the expected levels of population-level immunity.[9] Proxy measures for immunity were consistently found to be important factors associated with P(Detect). First, an increase in transmission during the past year was positively associated with the proportion of infections detected. In such settings, a higher proportion of susceptible individuals would be expected given the current estimated levels of transmission and these individuals therefore have an increased likelihood of becoming symptomatic.[3,21] Second, compared with non-African sites, African settings tended to have a lower P(Detect). Different global regions have had very different malaria histories, with malaria transmission in Africa being much higher for longer than in America or Asia, meaning that different levels of population immunity are expected.[4] Third, the interaction between prevalence and region in children further supports this notion, with the estimated P(Detect) in children generally being higher than in adults and remaining relatively constant across the range of estimated PCR prevalence. Children are less likely than adults to have acquired sufficient immunity to protect against symptoms and are therefore more likely to become sufficiently symptomatic to prompt care-seeking.[9] This theory is further reinforced by the slight increase in P(Detect) in African children as transmission intensity increases. This trend could represent opportunistically detected malaria infections in children acquiring sufficient levels of protective immunity earlier in life, meaning that the fever prompting care-seeking might not be causally related to their malaria infection.

The observed association is less straightforward when considering *P vivax*. The factors probably contributing to the negative association between P(Detect) and transmission intensity might be affected by several, non-mutually exclusive factors. First, *P vivax* infections typically have lower parasite densities than *P falciparum* infections.[22] Such lower parasite densities might be related to fewer infections being sufficiently symptomatic to prompt care-seeking and fewer infections that are expected to be infectious.[23] Furthermore, even if someone does seek care, the routine diagnostic tests for *P vivax* are currently less sensitive than those for *P falciparum*: thus, infections might not be confirmed even if infected individuals are presenting to a health-care facility and tested.[22] Next, although natural acquired immunity to *P vivax* is likely, the degree of the protection obtained and effect of hypnozoites on the probability that an infected individual will become symptomatic is not well understood. In areas with co-circulating parasite strains, efforts might also be biased towards *P falciparum*, which is traditionally the focus of malaria control and elimination programmes. The perception of risk for *P vivax* might differ to that for *P falciparum* infections, which alters care-seeking and diagnostic behaviours. Moreover, there might be sufficient cross-protection between the species, modifying the likelihood that an infection from either species would elicit symptoms.[24]

Across the settings examined, P(Detect) varied substantially, with health facilities in 27 clusters across six countries detecting at least half of all *P falciparum* infections and health facilities in nine clusters across three countries detecting at least half of all *P vivax* infections. Two factors that were consistently associated with a reduced probability that an infection will be detected within the health facility were the facility catchment population size and the transmission season. Intuitively, detecting one infection will be easier in 20 people than in 2000 people. Similarly, seasonality was defined according to the specific setting and parasite species. In the low transmission season, when vector densities are low, a malaria parasite might be more geared towards surviving until the next transmission season than causing clinical symptoms that could lead to treatment. The protective immunity acquired during the previous transmission season might also have not yet sufficiently waned for symptoms to develop, suggesting a lower probability of becoming symptomatic and therefore a lower estimated P(Detect).[25] It is also plausible that clinicians would be less likely to test for malaria or patients less likely to seek care for a fever when malaria is not perceived to be a problem.[26] These findings reinforce the view that good access to testing and treatment practices improves detection of malaria infections and a better understanding of malaria-attributable fevers could improve clinical algorithms that account for any seasonal changes in malaria presentation.[5,27,28] In settings where blood-stage malaria vaccines or other interventions reduce the likelihood of an individual becoming sufficiently symptomatic to seek care, the proportion of

infections detected within the health system will probably decrease.

Passive surveillance data for malaria are generally used for two purposes: resource allocation and monitoring trends in malaria.[29,30] From the resource allocation perspective, the magnitude of undercounting might not matter. The number of tests or malaria drugs to send to a health facility will not be affected unless additional people start to seek care or testing rates increase. Conversely, the effect on estimating current or changes in malaria burden could be substantial. It has long been assumed that, although within-catchment heterogeneity of transmission is not routinely represented, incident infections detected at the health facility are a good representation of transmission intensity in the community.[2,15] However, what is identified in the health system does not account for non-opportunistically detected new infections in the immune population or when an already infected person receives multiple inoculations with different strains of parasites.[31] Similarly, reductions in transmission intensity are not immediately detectable based on clinical incidence data. Once transmission declines, the first expected trend is a shift in the underlying age distribution of clinically detected cases, with older individuals presenting with clinical symptoms as immunity wanes in the population.[32,33] The prevalence of infected individuals, such as that assessed using easy access group surveys, and any change in the size of the parasite reservoir is arguably a more direct measure of progress in the short term, especially in a population with a large proportion of immune individuals.

The presence of undetected infections becomes especially critical in settings striving for and maintaining malaria elimination. A key factor initiating a shift to elimination strategies is routinely collected malaria data.[30] Allowing for the time for transmission to taper off naturally with corresponding decreases in population-level protective immunity, and improvements in health systems could be an option whereby relying on clinical data alone to detect all infections during the elimination phase might be sufficient. However, in settings accelerating elimination, the potential for any residual population immunity masking any asymptomatic or introduced infections must be acknowledged.[34] A better understanding of the probability that an infection becomes symptomatic and potentially detected by the health system will inform the critical point at which programmes could scale back control activities and rely on the health system to identify all infections (eg, P[Detect] of 100%). Until that point, maintaining diligent levels of control is essential.[35]

This study has some important limitations. It was a pooled analysis driven by a large number of clusters in a few countries and therefore was not powered to detect the specific change point whereby the majority of infections can be detected or the exact proportion of infections detected. The general trends observed are consistent with existing knowledge, and model fits were good despite the substantial variation in the data. The conclusions are nevertheless informative. Second, this was not a full systematic review and relied on secondary data analysis. We were unable to include all eligible community surveys because the ability to obtain matched PCD data was logistically unfeasible because of research sites being closed or the timeframe required to obtain local approvals was prohibitive. Some bias might have been introduced by the exclusion of some sites but the effect of this bias is expected to be non-differential and thus we believe our inferences are still valid. Similarly, because of the nature of this pooled analysis, not all variables of interest were available in all datasets, leading to derivation of the primary outcome variable and reliance on modelled or estimated covariates in some settings, potentially introducing some bias. However, the credible intervals were calculated accounting for this introduced uncertainty and the final model fits are consistent with known factors related to malaria transmission. Third, the more granular epidemiological considerations and differences between settings were not accounted for—for example, in southeast Asian settings, forest goers are known to be at increased risk of malaria infection due to the vectors' preferred ecological niche, leading to a different risk profile in this setting.[36] Accounting for the differential risk profiles can help make health systems more effective at detecting infections by adapting activities to where they are more likely to find them. Moreover, cases might not report to their nearest health facility, might seek care at private facilities, or be misclassified as imported or local. However, people from other catchments might also prefer to attend the included facility, resulting in non-differential misclassification of infections or cases. Similarly, travel history is used to define an infection as imported. Classification is generally improving, especially in low transmission settings where this is more relevant, but variation exists at both the facility and country level in how imported was classified. The data generated at the facility level are what is available for decision making so, although the data used here might not be perfect, we expect the resulting inferences to be valid. Some care-seeking malaria infections might have been misclassified because of the low diagnostic sensitivity of rapid diagnostic tests or routine microscopy, which is again lower than that of molecular methods used to define the extent of the infected population in the community. The aim here was to show the degree of bias when relying on routinely collected data to estimate the magnitude of malaria burden and not a direct comparison of two populations using different diagnostic tools. Finally, the *P vivax* data available were cross-sectional. Infections detected might be due to relapse instead of being an incident infection. However, in terms of P(Detect), this is expected to be a non-differential bias and unlikely to affect the observed trends.

This study has confirmed that health facilities detect a small proportion of the malaria parasite reservoir, with

routine data underestimating transmission intensity and the magnitude of malaria-infected populations. When transmission is very low, health facilities become more effective at detecting infections, and this finding is observed for both *P falciparum* and *P vivax*. Promoting better health-seeking behaviour of infected individuals and investing in better access to care for testing would lead to more infections being detected and, along with the iterative approach of surveillance as an intervention as outlined by the WHO, might ultimately contribute to accelerating malaria elimination.

Contributors

GS conceived and designed the study. GS, KF, LG, JuM, JA, JoM, DJB, TPE, JaM, PJL, MLM, FEE, FT, JCS, AMQ, AS, ML, SY, SS, EP, JG, KEH, AY, JFL, MAC, KP, MM, JL, DMP, LVS, FN, GD, AD, TB, UD'A, and CD were involved in the primary data collection in the different study sites and facilitated access to the health systems malaria data where needed. EC, KB, and PG provided access to the covariables from the Malaria Atlas Project data. GS and NS had access to and analysed the data. All authors participated in the development and provided a critical review of the reported research. All authors approved the final report for the publication and are accountable for the accuracy and integrity of the work.

Declaration of interests

We declare no competing interests.

Data sharing

The anonymised, aggregated data collected to support this pooled data analysis are available from the corresponding author upon reasonable request.

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